For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
universitatis
albertaensis

University of A

Digitized by the Internet Archive
in 2019 with funding from
University of Alberta Libraries

https://archive.org/details/Young1962

esis
962(F)
#78

THE UNIVERSITY OF ALBERTA

CELL WALL METABOLISM AND GROWTH OF ETIOLATED PEA EPICOTYLS

by

MURRAY RONALD YOUNG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

JULY, 1962

UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Cell Wall Metabolism and Growth of Etiolated Pea Epicotyls submitted by Murray Ronald Young in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

1. Sections from the apical region of the third internode of etiolated pea epicotyls were incubated on water, and a loss of ethanol- and ether-insoluble materials was shown to occur in those sections which grew. Those sections which matured but did not grow gained in insoluble dry weight.

2. Sections were incubated on 2% ^{14}C-sucrose and determinations were made of the amounts and kinds of radioactive wall components that were synthesized. The main products were polymers of galacturonic acid, galactose, arabinose, xylose, and glucose, *i.e.*, pectic materials, hemicelluloses, and cellulose.

3. Pectic materials accounted for approximately 30% of the radioactivity in the wall in all sections. In sections which grew, hemicelluloses accounted for a larger part of the radioactivity (45%) than in sections which did not grow(35%). More cellulose was formed in maturing sections (35%) than in growing sections(25%).

4. In growing sections, the increments in insoluble dry weight were not as great as the weight of radioactive sucrose incorporated into wall materials. Thus part of the initial insoluble material dissolved during the experiment.

Reasons are given for concluding that this indicated the breakdown of wall materials.

5. In maturing sections, the increments in insoluble dry weight more than accounted for the radioactive sucrose incorporated into the wall. Little or no wall breakdown appeared to occur.

6. Since cell wall catabolism and the synthesis of hemicelluloses took place more extensively in growing than in maturing sections, these phenomena may be related to the greater plasticity of enlarging cells.

ACKNOWLEDGMENTS

I am deeply indebted to Dr. G. A. Maclachlan for his guidance and encouragement throughout the course of this investigation and for his generous help in preparing this manuscript. I would also like to thank Dr. R. G. H. Cormack for instruction in the preparation of slides for photomicroscopy and for his helpful advice. All photography was carried out with the expert assistance of Mr. C. T. Duda.

TABLE OF CONTENTS

Page

INTRODUCTION 1

LITERATURE REVIEW 3

MATERIALS 10

METHODS 15

1. Experimental Conditions 15

2. Quantitative Determinations 17

3. Extraction Procedures 20

A. Detached Epicotyls 20
B. Sectioned Third Internodes 21
Ethanol- and ether-soluble fraction 21
Na_2EDTA-soluble fraction 21
HCl-soluble fraction 22
H_2SO_4-soluble fraction 22

RESULTS 24

1. Growth and Metabolism of Detached Epicotyls.. 24
A. Determination of the Growing Region 24
B. Incorporation of ^{14}C-sucrose 24

2. Growth and Metabolism of Sectioned Epicotyls on Water 30

3. Growth and Metabolism of Sectioned Epicotyls on Sugar 33
A. Experiment 1 33
(i) Growth 33
(ii) Incorporation of ^{14}C-sucrose 33
(iii) Weight Changes 39
B. Experiment 2 42

DISCUSSION 47

BIBLIOGRAPHY... 53

APPENDIX... 61

LIST OF TABLES

Page

I GROWTH AND UPTAKE OF ^{14}C-SUCROSE BY DETACHED EPICOTYLS.................................... 27

II WEIGHTS OF PEA EPICOTYL SECTIONS FLOATED ON WATER FOR 48 HOURS.................................... 31

III PERCENTAGE CHANGE IN LENGTH AND WEIGHT OF SECTIONS INCUBATED ON WATER FOR 48 HOURS........................ 32

IV PERCENTAGE INCREASE IN LENGTH AND WEIGHT OF SECTIONS INCUBATED ON 2% SUCROSE FOR 24 HOURS................... 34

V RADIOACTIVITY INCORPORATED INTO MATERIAL INSOLUBLE IN ETHANOL AND ETHER.................................. 35

VI RADIOACTIVITY INCORPORATED INTO COMPONENTS OF THE HCl-SOLUBLE FRACTION.................................. 40

VII COMPARISON OF THE WEIGHT INCREASES OF INSOLUBLE MATERIAL WITH THE WEIGHT INCREASES DUE TO ^{14}C-SUCROSE INCORPORATION.................................. 41

VIII WALL SYNTHESIS FROM ^{14}C-SUCROSE AND BREAKDOWN OF THE PRE-EXISTING INSOLUBLE MATERIALS................... 44

IX WALL SYNTHESIS AND BREAKDOWN OF INSOLUBLE MATERIALS, TAKING INTO ACCOUNT POSSIBLE ADDITIONAL SYNTHESIS FROM UNLABELLED SUBSTRATES.................................. 45

X SYNTHESIS OF INSOLUBLE MATERIALS, EXPRESSED AS PERCENTAGE OF INITIAL INSOLUBLE MATERIAL............... 48

LIST OF FIGURES

Page

IA. Sections of the third internode of the etiolated pea epicotyl used for experimentation.................. 13

1B. Photomicrographs of regions cut transversely through the basal end of the hook and the approximate centres of the five internodes.................. 14

2. Apparatus for incubating intact third internodes..... 16

3. Unknown radioactive substances co-chromatographed and chromatographed in parallel with authentic arabinose and xylose.............................. 19

4. Procedure for the extraction of sectioned epicotyls.. 21a

5. Elongation of regions along the epicotyl shaft of intact third internodes.......................... 25

6. Radioautographs of epicotyls grown on ^{14}C-sucrose.... 28

7. Radioautographs of chromatographs of ethanol-soluble fractions extracted from epicotyls growing on ^{14}C-sucrose............................... 29

8. Chromatographs of hydrolysates derived from section one of Na_2EDTA-soluble, HCl-soluble, and H_2SO_4-soluble fractions.......................... 37

9. Radioautographs from chromatographs of hydrolysates of Na_2EDTA-soluble, HCl-soluble, and H_2SO_4-soluble fractions.................................. 38

INTRODUCTION

It was first pointed out over thirty years ago by Heyn (see his review, 1940), that growth of plant cells is accompanied by a permanent increase in the area of the wall. He referred to this irreversible increase in wall as "plastic extension". There may also be a reversible stretching of wall fibres, or "elastic extension", but this can occur in response to osmotic changes in many types of cell and can not be regarded as growth. Thus Heyn suggested that since wall plasticisation appears to be a property of growing tissues, it may regulate or limit the ability of plant cells to enlarge.

The nature of the wall changes which lead to plasticisation and growth are not well understood. Nevertheless, it is widely agreed that irreversible wall extension requires a synthesis of new wall materials. A number of theories have appeared to explain the mechanism of wall deposition and there have been several studies recently on the changes in wall composition during growth. It is also realised that plastic extension would require a "loosening" of the interwoven structure of the pre-existing wall. This has usually been studied as a physical phenomenon involving only the re-orientation of fibres by stretching or sliding. The possibility that it may be a biochemical phenomenon involving a breakage of bonds in the old wall has received little attention.

The present study deals with the synthesis and breakdown of wall materials (metabolic turnover) in sections cut from growing and non-growing regions of the etiolated epicotyl of *Pisum sativum* L. Two types of experiments were carried out to test for loss of insoluble dry weight, as a measure of wall dissolution and bond breakage. In the first, sections were incubated on water and measurements were made of growth and changes in wall weight. In the second, sections were incubated on solutions of radioactive sucrose and analyses were made of the amounts and kinds of wall materials that were synthesised from the substrate. Comparisons were made between the weight of radioactive sucrose incorporated into wall materials and the increment in insoluble dry weight. In sections where wall synthesis was greater than weight increment, a dissolution of initial insoluble materials was implied. Accordingly, the experiments yielded information on the relationships between growth and both anabolism and catabolism of wall materials.

LITERATURE REVIEW

The structure and composition of plant cell walls is discussed in detail in the text by Roelofsen (1959) and reviewed by Frey-Wyssling (1957), Preston (1958), and Northcote (1958). The relationships that have been extablished between cell wall changes and growth are treated fully by Setterfield and Bayley (1961) and Wardrop (1962) and considered briefly in reviews of growth and auxin action by Galston and Purves (1960) and Bonner (1961).

The present review deals chiefly with recent publications on the biochemical changes in the walls of growing cells. Discussion will be limited to primary cell walls.

1. Theories of Wall Deposition.

A number of theoretical possibilities have been proposed to explain the mechanism whereby extension of cell walls may be brought about by the deposition of new material.

On the basis of an electron microscopic study of the walls of cells of Avena coleoptiles, Mühlethaler (1950) suggested that extension occurs by "bipolar tip" growth. He proposed that the protoplasm extends through the tips of cells depositing new wall material as it advances. This explanation is now accepted for elongation of root hairs (Cormack, 1961) and cambial initials (Bannan, 1956). However, it does not appear to explain the growth of other

types of cell. For example, examination of the distribution of pits in the parenchyma cells of Avena coleoptiles by Wardrop (1955) and in the cortical cells of onion root tips by Scott et al., (1956) provided strong evidence that growth occurs over the whole length of these cells. In both cases, the number of pit fields of the primary wall increases during elongation, apparently as a result of the division of pre-existing pits. It was suggested that thin strands of cellulose are deposited across the pit, gradually widening and increasing the distance between the two halves. This mechanism would bring about elongation of the whole cell by deposition in localized parts of it. Localized deposition was also proposed by Preston and Ripley (1954) who observed proteinaceous masses, interpreted as "islands of synthesis", distributed on the surface of walls of conifer cambium cells. Bayley et al., (1957) suggested that these were simply fragments of protoplasm displaced from plasmodesmata during preparation of the cells for electron microscopy.

None of these models for wall deposition agrees with recent observations by Setterfield and Bayley (1959) which show directly the location of synthesis on and in the old wall. Radioautographs were prepared of squashed walls from coleoptile segments grown on a tritiated sucrose solution, and these show that wall deposition is not localized at a few centres but occurs over the whole area

of the wall. Deposition may be concentrated on the inner surface of the walls of some cells. It is therefore necessary to reject the theories of growth by "bipolar tip", "pit field", and "islands of synthesis". The radioautographs lend support, however, to the early views of Frey-Wyssling and Stecher (1951) who proposed a theory of "mosaic" growth in which new material is laid down evenly by intussusception throughout the entire thickness and area of the wall. In cases where new wall material is deposited mainly by apposition, a theory of "multi-net" growth, as proposed by Roelofsen and Houwink (1953) would more nearly apply. In this theory, the wall would be built up in a series of layers or multi-nets by continuous deposition on the inner surface. Such a layering has been observed with the electron microscope by Wardrop (1956), Green (1960), and Setterfield and Bayley (1957). These authors agree that the orientation on microfibrils in the wall changes from transverse on the inner surface to longitudinal towards the outside.

Many of the theories of the mechanism of wall deposition have been proposed with the understanding that wall synthesis is the cause of cell enlargement. In fact, there is only evidence that wall synthesis accompanies and is necessary for continued cell growth. Most investigators have observed an increase of wall weight during growth (Christiansen and Thimann, 1950; Setterfield and Bayley, 1957; Brown, 1959; Bonner, 1961), but no clear relationship

has been found between the rate of cell enlargement and the net increase in weight of wall materials. In an early study, Bonner (1934) showed that oat coleoptiles grew slowly at 2°C with no detectable increase in wall weight. There is even one report (Solberg and Higinbotham, 1957) of growth of pea epicotyls on water for a six-day period where there was a concurrent loss of dry matter, part of which may have been wall. It was concluded by Setterfield and Bayley (1961) that although growth and wall deposition are closely related they do not always remain perfectly in step.

Analyses of the chemical composition of the primary cell wall have not revealed any change which is consistently associated with cell elongation. This is perhaps not surprising in view of the wide differences in the relative amounts of the various wall components of the primary wall as measured in different species (Setterfield and Bayley, 1961). Cellulose has been recorded as making up 20-50% of the wall, hemicellulose 4-50%, pectin 1-50%, protein 2-30% and lipid 1-25%. During growth the amounts of all of these components are usually found to increase (Jensen and Ashton, 1960; Bishop _et al._, 1958; Brown, 1959). If radicactive sugars are supplied during growth, label is incorporated into all major wall polysaccharides (Brown and Neish, 1954; Ordin and Bonner, 1957; Matchett and Nance, 1962). Other precursors are readily incorporated into protein (Boroughs and Bonner, 1953),lipid (Nance,1958), and pectin (Ordin

et al., 1957). It is logical to expect that prolonged and irreversible wall extension requires the deposition of new wall, but there is no evidence that the rate or amount of any one wall component of group of components is proportional to the rate or amount of growth.

2. Theories of Wall Loosening.

Since the first electron micrographs of the structure of the cell wall appeared it has been realized that extension in surface area would require the re-orientation of fibres (Frey-Wyssling, 1952). Nevertheless, it is difficult to imagine that a simple stretching or sliding of fibres could accomplish extension of such an encrusted and interwoven structure. It was therefore suggested by Thimann as early as 1954 that wall extension probably requires a partial dissolution of wall fibres. This phenomenon has since been referred to in such vague terms as "loosening" (Ray, 1961), "relaxing" (Galston and Purves, 1960), "softening" (Bennet-Clark, 1961) and "increased wall flexibility" (Adamson and Adamson,1959).

Theoretically, a loosening of the wall would have to occur before extension could take place and evidence is accumulating that this is, in fact, the case. The flexibility of growing tissues has been repeatedly demonstrated by measuring the degree to which tissue can be

made to bend or extend by physical means (Heyn, 1940). Extension and subsequent contraction give a measure of the reversible (elastic) and irreversible (plastic) flexibility of the tissues. It has been found by varying environmental conditions and adding growth hormones that the plasticity of the wall is proportional to the rate of growth. For example, when tissue is placed in hypertonic solutions of mannitol, its cells do not enlarge or lay down new wall material, even in the presence of auxin (Bayley and Setterfield, 1957). However, if the tissue is then placed in water, expansion resumes, especially when auxin is present during the pre-treatment (Thimann, 1954). Aerobic pre-treatment with auxin in mannitol will even permit subsequent expansion in water under anaerobic conditions (Cleland and Bonner, 1956). Evidently auxin can induce a loosening of the wall without wall deposition and before cell expansion.

The problem remaining is to explain the mechanism of the loosening process. The only coherent explanation that has been advanced to-date is usually referred to as the "pectin theory". It has been shown that divalent ions, particularly calcium, inhibit growth. Carlier and Buffel (1955), Bennet-Clark (1956), and Tagawa and Bonner (1957), have suggested that this inhibition is due to increased ionic binding between pectic carboxyl groups. Since calcium pectate is a gel _in vitro_, it is expected to contribute to

wall rigidity and inhibit extension *in vivo*. In support of this proposal, it is pointed out that low concentrations of chelating agents stimulate growth (Heath and Clark, 1956), supposedly by removing calcium and thereby reducing wall rigidity. Moreover, pectinase solutions cause severe swelling and deformity of root hairs and hair-forming cells growing in calcium solutions (Cormack,1956). In an extension of the theory, Bryan and Newcombe (1954), and Glaziou (1959), have proposed that the action of pectin methylesterase (PME), by reducing the number of free carboxyl groups, would reduce calcium esterification and thus loosen the wall.

The pectin theory has been critisized recently on the grounds that both the pectin (Bishop *et al*., 1958) and calcium (Carr and Ng, 1959) contents of the wall appear to be too low to provide the degree of rigidity envisaged. Auxin does not influence PME activity (Jansen *et al*., 1960; Bennet-Clark , 1961) or the amount or distribution of Ca^{45} in growing walls (Cleland, 1960). In this connection, Setterfield and Bayley (1961) conclude that "although the hypothesis that pectic substances determine the plasticity of the wall is attractive, it remains unsubstantiated".

MATERIALS

Since the now classical studies on auxin bioassay (Went and Thimann,1937), the apical region of the third internode of etiolated pea epicotyls has been perhaps the most commonly used tissue for investigation of stem growth. All present experiments were carried out with this material. Seeds of Pisum sativum L. var. Alaska, were supplied by Pike Seeds Ltd., Edmonton, and grown by the Sunset Seed Co., Armstrong, B. C.

A standard technique was used for growing the peas. Seeds were soaked in 1% Javex for one hour and washed in running distilled water for three hours. During this period, those seeds that absorbed water very rapidly or very slowly were discarded. The remaining seeds were sown in the artificial soil "Perlite", which is an exploded volcanic rock containing high percentages of Si and Al and very low percentages of soluble salts. Two litres of Hoagland's solution #1 and six litres Perlite were placed in polyethylene flats. The surface of the wet Perlite was covered with seeds which were in turn buried under a thin layer of dry Perlite. The flats were incubated in the dark at 26±1°C. and all further handling was done under dim green light. An additional 1.5 litres of dry Perlite was added on the third day of incubation and 0.5 litres of distilled water was added on the third and fifth days of incubation. Plants were ready for harvesting after

7-9 days growth. They had developed third internodes and those 30-50 mm long were selected for experimentation.

Figure 1 illustrates the gross and microscopic anatomy of the hook region and the sub-apical 25 mm of the third internode. For most experiments, the internode shaft was cut into five successive five millimetre sections (numbered from the apex one to five as illustrated). Slides were prepared for microscopy, according to standard procedures (see Appendix 1), from the central region of the sections they represented.

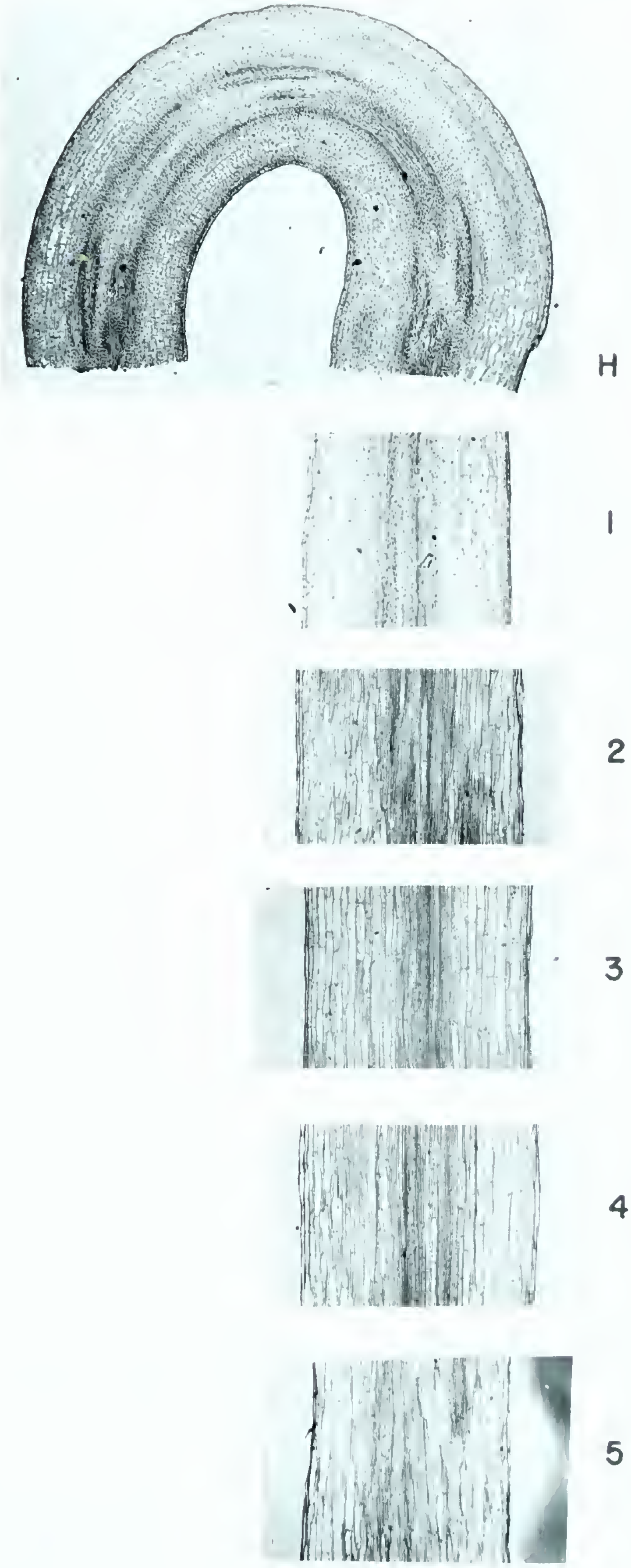
Figure 1A illustrates the anatomy of the experimental tissue in longitudinal view. Cell division is confined to the hook region and the apical part of section one. Cell elongation commences in the basal part of the hook and ceases approximately 10 mm below (section two). Vascular tissue is found throughout the hook region and in fact, continues into the plumule. Annular and spiral vessel elements are found in all sections. Staining with safranin did not produce any evidence indicating lignin formation and there is no secondary tissue, even in section five.

In Figure 1B, cross-sections of the tissure are shown. No indication of cell division could be found in section two. Cell diameter reaches its maximum in section three. Differentiation appears to be relatively complete when cell enlargement begins. Of the four cortical bundles,

two are vascular and two are fibrous (Hayward,1938).

It should be noted that the transition zone in _Pisum_ _sativum_ is very long and the anatomy shown in Figure 1 is not typical of the stem. A definite stem pattern is not laid down until the fourth internode (Hayward, 1938).

Figure 1A. Sections of the third internode of the etiolated pea epicotyl used for experimentation. Diagram illustrates the approximate shape and dimensions of the hook (H) and five subapical five mm sections of the epicotyl shaft; the second node lies just below the fifth section. Photomicrographs show regions cut longitudinally through the approximate centre of the hook and five sections; slides were stained with fast green and safranin as in Appendix 1; magnification x 22.


H
1
2
3
4
5

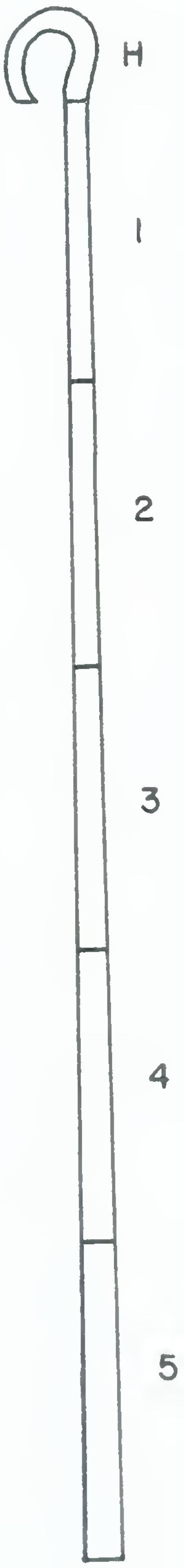
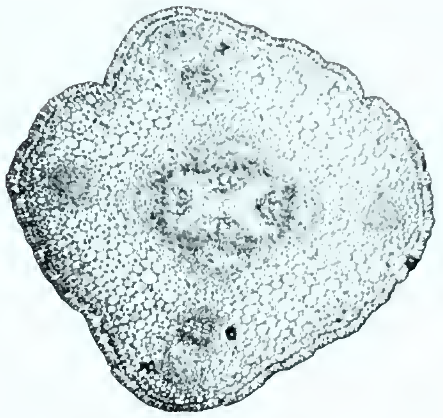
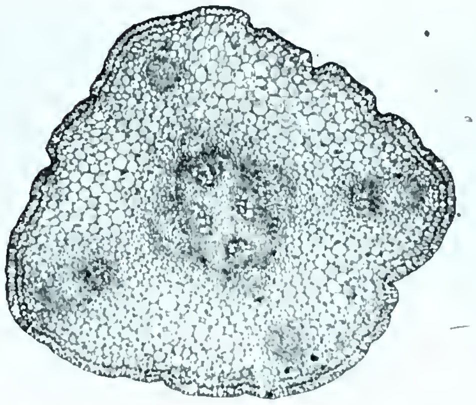

H
1
2
3
4
5

Figure 1B. Photomicrographs of regions cut transversely through the basal end of the hook and the approximate centres of the five internodal sections (see Figure 1A); magnification x 35.

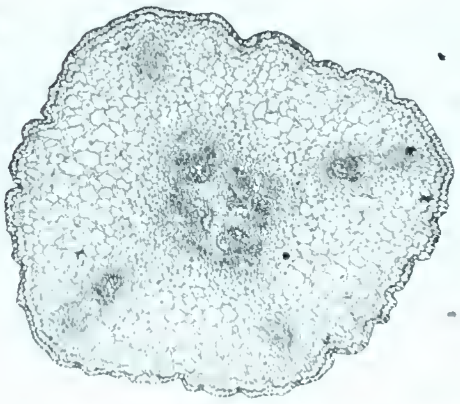
H



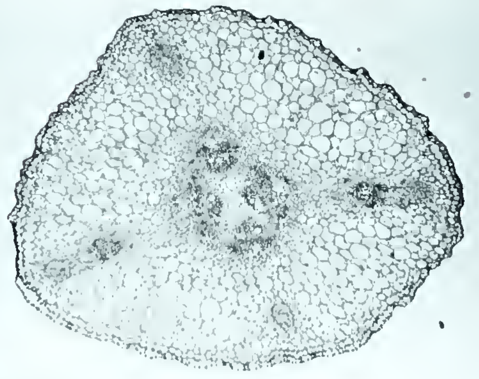
1



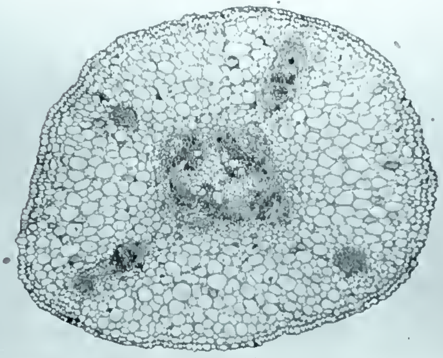
2



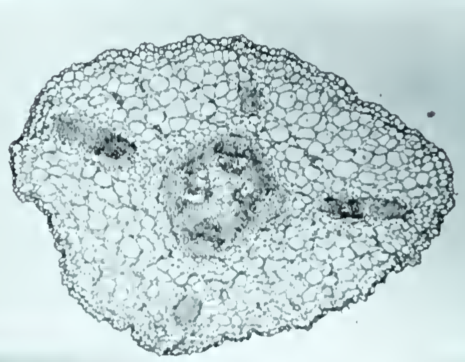
3



4



5



METHODS

1. Experimental Conditions.

During all experiments, intact or sectioned epicotyls were handled under dim green light and incubated in the dark at 26±1°C.

For preliminary experiments, the apical region of the third internode was incubated with the plumule, hook, and epicotyl shaft intact in an assemblage illustrated in Figure 2. The shaft was cut with a razor blade in a simple guillotine at either 30 or 40 mm below the arch of the hook. The bases were inserted through holes in a lucite disc which, in turn, was resting on short legs on wet filter paper in a seven cm petri dish. The epicotyls were held in an upright position by this apparatus. Two ml 2% sucrose were supplied in each dish. The epicotyls grew for several days without wilting, provided the whole assemblage was enclosed in a glass jar.

For major experiments, the hook and plumule were removed from the third internode with a razor blade and the apical region of the internode shaft was cut into five mm sections as shown in Figure 1. Usually 40 sections from each region were incubated in 25 ml erlenmeyer flasks on 1.5 ml of either distilled water or 2% sucrose. This was sufficient to half-submerge the sections. When CO_2 determinations were required, 75 ml erlenmeyer flasks were used

Figure 2. Apparatus for incubating intact third internodes.

and CO_2-free 1N KOH was added to their centre wells. During experiments, flasks were closed by rubber serum-bottle caps.

2. Quantitative Determinations.

All weight measurements were made on a Mettler B Gramatic Balance, Model 6, which measures to five decimal places with a reproducibility of 0.02 mgm. Fresh weights were estimated to the nearest mgm immediately after blotting excess moisture from the tissue surfaces. Dry weights were estimated to the nearest 0.01 mgm and corrected to the nearest 0.1 mgm.

Length measurements of both intact and sectioned internodes were carried out to the nearest 0.5 mm with a ruler.

Respired CO_2 was determined by back titration of KOH with HCl in the presence of $BaCl_2$. Care was taken to ensure that the KOH was initially CO_2-free and that contamination by atmospheric CO_2 during handling was minimized. The $BaCO_3$ precipitate was transferred to centrifuge tubes and washed with ethanol. It was preserved under ethanol for further analysis of radioactive content.

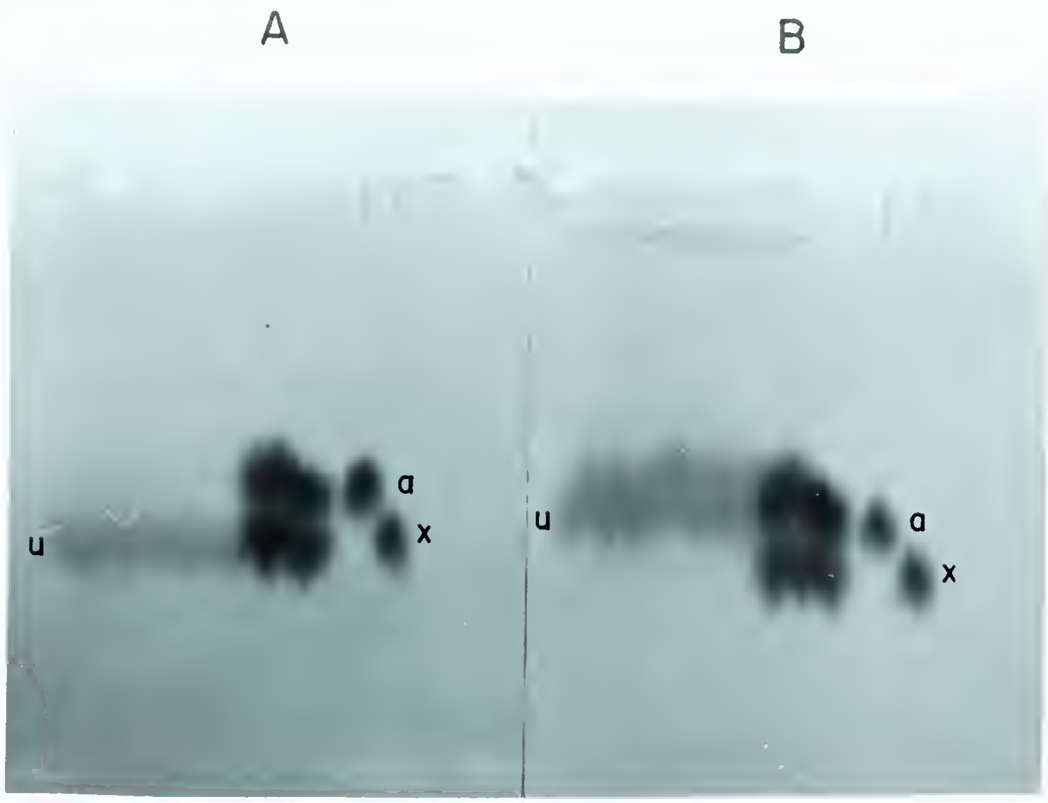
For the separation of components of alcoholic extracts and acid hydrolysates, descending chromatography on Whatman #1 paper was used. Radioautography delineated radioactive areas on the paper. These areas were cut out and quantitatively eluted by the method of Porter and May (1955).

Solvents found to be the most suitable for separating sugars were the butanol-rich phase of n-butanol:acetic acid: water (4:1:5 v/v) and n-propanol:ethylacetate:water (7:1:2 v/v). Development times for these solvents were 44 and 24 hours respectively. In order to identify major components of the hydrolysates on chromatographs, they were revealed by radioautography, eluted and re-chromatographed along with known substances of similar Rf values. The Rf values for unknown and known substances were compared after spraying with the appropriate reagents. Spray reagents used for revealing sugars, amino acids, and organic acids are described by Block *et al.*, (1957). Typical results which led to the identification of arabinose and xylose are shown in Figure 3.

For radioautography, Kodak No-Screen Medical X-Ray Film was placed in close apposition to chromatographs or dried and flattened tissue and enclosed in light-tight holders. The time necessary to produce a satisfactory image was determined empirically. Extracted epicotyls were prepared for radioautography by arranging them on the adhesive surface of masking tape fixed to a piece of stiff cardboard. The tape not covered by the internodes was dusted with infusorial earth. The epicotyls were then pressed under blotting paper and allowed to dry.

Radioactivity in the various samples was measured as follows. Aliquots of liquid samples were transferred to

Figure 3. Unknown (u) radioactive substances co-chromatographed and chromatographed in parallel with authentic arabinose (a) and xylose (x). Solvent: butanol:acetic acid:water; spray reagent: benzidine·HCl; Unknowns derived by elution from chromatographs of hydrolysates of wall materials extracted from pea epicotyls growing on ^{14}C-sucrose. Note unknown A is xylose; unknown B is arabinose.


A
B
u
a
x
u
a
x

aluminum discs (1.25" in diameter) and spread evenly by either ethanol or a wetting agent. The samples were then dried and counted in air, under lead, 4.5 mm from the end window of a Geiger-Mueller Detector with a Nuclear Chicago Decade Scaler (Model 186). Weighed aliquots of solid samples, e.g., $BaCO_3$ and insoluble residues, in alcoholic slurries were spread as evenly as possible onto the discs. All samples were counted at infinite thinness. The range of proportionality between weight increment and cpm lay below five mgm. With this apparatus, one microcurie of ^{14}C-sucrose yielded 59,000 cpm for experiments reported in Results, section one. Subsequently, the geiger-tube was moved and a new value of 66,400 cpm/ microcurie was used for experiments reported in Results, section 2.

3. Extraction Procedures.

A. Detached Epicotyls.

In preparation for radioautography of internodes after growth on radioactive sugar, the ethanol- and ether-soluble materials were removed as described below (see page 21). Further extractions were made with either dilute alkali or acid. The fraction soluble in hot 5% NaOH after four hours was expected to include protein, pectic materials and polyuronide hemicelluloses (Bonner, 1950). Extraction with hot 1N HCl (see page 22) was expected to remove similar substances, leaving behind only cellulose, lignin, and

the more stable hemicelluloses. Since internodes after alkali treatment disintegrated more readily than those after acid treatment, the latter may not have removed as much of the cementing material.

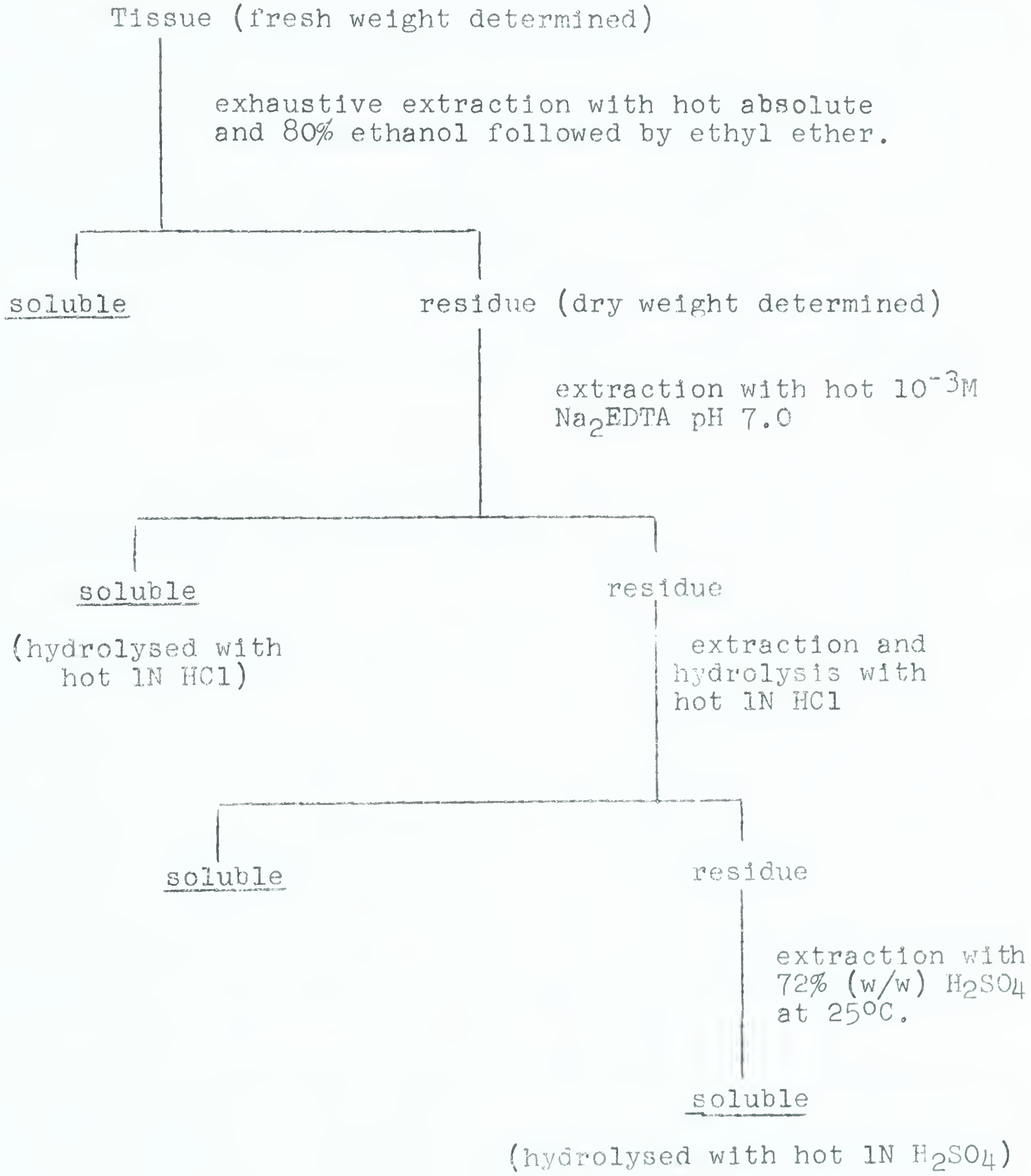
B. Sectioned Third Internodes.

An outline of the procedure used for extracting sections is given in Figure 4.

Ethanol- and ether-soluble fraction: Sections were extracted without grinding for five half-hour periods with 15 ml hot 80% ethanol alternated with five half-hour periods with 15 ml hot absolute ethanol, followed by a two-day extraction in ethyl ether. The combined extracts were taken to dryness over $CaCl_2$ *in vacuo* at room temperature and analysed quantitatively by chromatography. This treatment was expected to remove free sugars, amino acids, organic acids and lipids.

Na_2EDTA-soluble fraction: The ethanol- and ether-extracted material was dried and ground to a powder in test tubes with a glass pestle before and after addition of two ml 10^{-3}M Disodium Ethylenediaminetetraacetate (Na_2EDTA). The tubes were heated on a boiling water bath for 30 minutes and their contents quantitatively transferred to tubes for centrifugation. Extraction was repeated and the combined

Figure 4. Procedure for the extraction of sectioned epicotyls.


Tissue (fresh weight determined)
exhaustive extraction with hot absolute and 80% ethanol followed by ethyl ether.
soluble
residue (dry weight determined)
extraction with hot 10^{-3}M Na_2EDTA pH 7.0
soluble
(hydrolysed with hot 1N HCl)
residue
extraction and hydrolysis with hot 1N HCl
soluble
residue
extraction with 72% (w/w) H_2SO_4 at 25°C.
soluble
(hydrolysed with hot 1N H_2SO_4)

extracts were made 1N with respect to HCl. Hydrolysis was carried out in sealed test tubes for three hours on a boiling water bath. The hydrolysate was taken to dryness in vacuo over $CaCl_2$ and the HCl was removed by distillation into NaOH in the desiccator. The components were analysed by chromatography. Hot solutions of Na_2EDTA were expected to remove all pectic materials, including Ca-pectate (Kertesz, 1951), and low molecular weight hemicelluloses,if present (Jermyn, 1955).

HCl-soluble fraction: The residues after Na_2-EDTA extraction were suspended in 1N HCl in sealed tubes and heated on a boiling water bath for three hours. Insoluble material was removed by centrifugation and re-extracted with HCl. The combined extracts were taken to dryness in vacuo over $CaCl_2$ and NaOH and analysed by chromatography. This treatment was expected to remove and hydrolyse much of the protein and hemicellulose. The standard definition of hemicellulose is given by Gascoigne and Gascoigne (1960) as "cell wall polysaccharides removable by extraction with hot or cold dilute alkali and which can be hydrolysed with dilute acids to give the constituent monosaccharide units". Alkali extraction was omitted from the present procedures because of the difficulty of removing KOH from samples to be analysed by chromatography.

H_2SO_4-soluble fraction: Cellulose was extracted and hydrolysed by a standard method as refined by Mortimer and Wylan (1962). The residues after HCl extraction were

suspended in 72% (w/w) H_2SO_4 for two hours at room temperature. The acid was then diluted to 1N and the mixture refluxed for three hours. This completely dissolved the residue. The hydrolysate was then neutralized with $Ba(OH)_2$ and the $BaSO_4$ precipitate removed by centrifugation and washed with ethanol. The hydrolysate and washings were combined and taken to dryness over $CaCl_2$ and analysed by chromatography.

RESULTS

1. Growth and Metabolism of Detached Epicotyls.

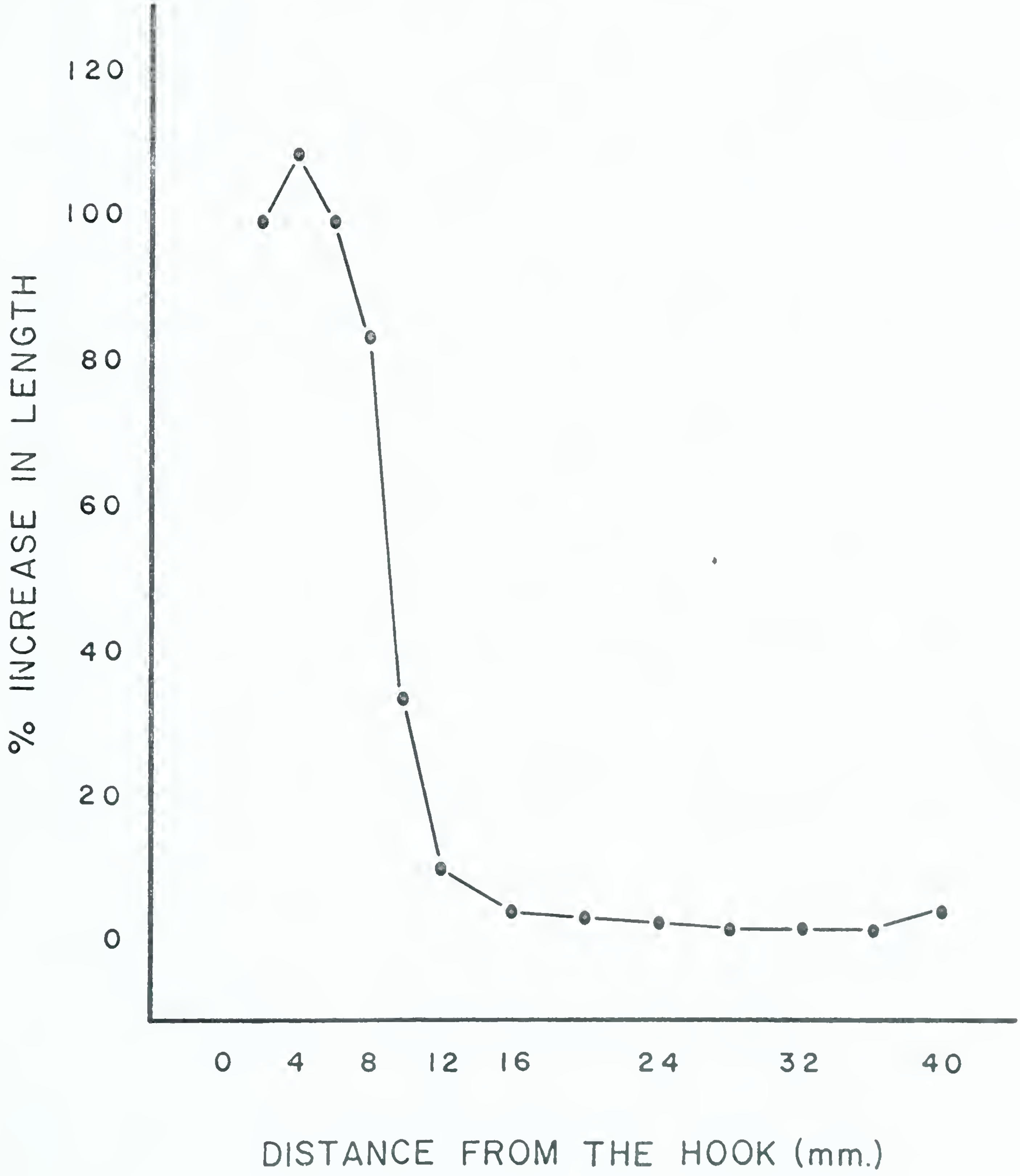
A. Determination of the Growing Region.

Epicotyls bearing third internodes were cut 40 mm below the hook and the epicotyl shaft was gently pressed against a marking apparatus which left lanolin-charcoal delineations two or four mm apart. The epicotyls were allowed to grow with their bases in 2% sucrose (see Figure 2) and at the end of 24 hours the average distances between delineations were determined. The results are recorded in detail in Appendix 2 and the percentage increases in length of the various regions are illustrated in Figure 5. The apical six mm region approximately doubled in length whereas the basal 30 mm showed little or no elongation. This growth pattern was expected on the basis of microscopic examination (see Figure 1) since parenchyma cells originate in the hook region and reach maximum length about 10 mm below.

B. Incorporation of ^{14}C-sucrose.

Sixty-four epicotyls were cut 30 mm below the hook and incubated with their bases in 1% ^{14}C-sucrose (specific activity 66,000 cpm/mgm). At 15 time intervals over a 16 hour period, four internodes were removed and analysed for growth and distribution of radioactivity. The lengths

Figure 5. Elongation of regions along the epicotyl shaft of intact third internodes. Epicotyls were cut four cm below the hook and incubated with bases in 2% sucrose for 24 hours.


% INCREASE IN LENGTH
120
100
80
60
40
20
0
0
4
8
12
16
24
32
40
DISTANCE FROM THE HOOK (mm.)

achieved and the amounts of radioactivity incorporated are recorded in Table 1. The ethanol-soluble fractions were chromatographed and radioautographs of these are shown in Figure 7. Figure 6 shows radioautographs made of the internodes themselves after they had been extracted with ethanol (Figure 6A), with ethanol followed by 5% NaOH (Figure 6B) and with ethanol followed by 1N HCl (Figure 6C).

The epicotyls elongated at a rate of about 0.5 mm per hour for the first eight hours after which their rate of growth declined (Table 1). The amount of radioactivity in the ethanol-soluble fraction increased linearly with time until about 10 hours, after which it appeared to approach an equilibrium value. Presumably this value was just sufficient to balance the rate of withdrawl of soluble ^{14}C for respiration and the synthesis of insoluble materials. The major radioactive components of the soluble fraction were identified (Figure 7) as sucrose, glucose, and fructose. These were present in the epicotyls in the approximate ratio of 2:1:1 throughout the experiment. Other radioactive products were identified as amino and organic acids by reaction with the appropriate spray reagents. Radioautographs of the extracted internodes (Figure 6) showed that radioactivity was incorporated into the insoluble tissue material continuously throughout the experiment. Even within the first hour of incubation, the complete length of the internodes had become radioactive, indicating a very rapid translocation of

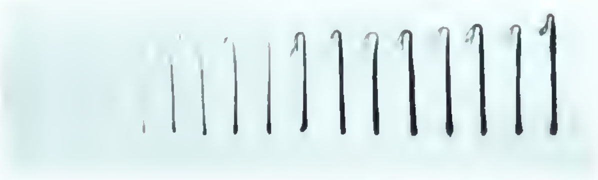
TABLE 1

GROWTH AND UPTAKE OF ^{14}C-SUCROSE BY DETACHED EPICOTYLS

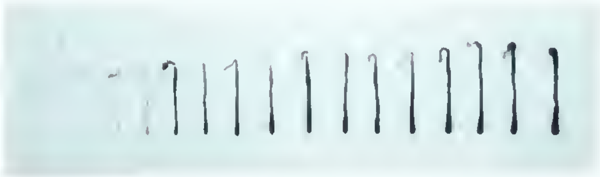
Time (hours)	Average length (mm) (mean of 4 replicas)	Ethanol-soluble fraction (cpm/4 epicotyls)
0.25	30.0	102
0.50	30.0	142
0.75	30.1	212
1.00	30.7	247
2.00	31.5	459
3.00	32.4	581
4.00	32.8	1096
5.00	32.9	891
6.00	33.9	1051
7.00	33.6	1222
8.00	33.8	1357
10.00	34.1	1533
12.00	35.0	1601
14.00	35.4	1694
16.00	35.2	1742

Figure 6. Radioautographs of epicotyls grown on ^{14}C-sucrose. Samples from left to right were harvested at the time intervals indicated in Table I. A - ethanol- and ether-extracted; B - ethanol-, ether- and 5% NaOH-extracted; C - ethanol-, ether- and 1N HCl-extracted. Magnification x 1/3

A



B



C

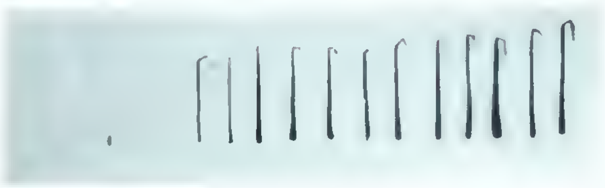
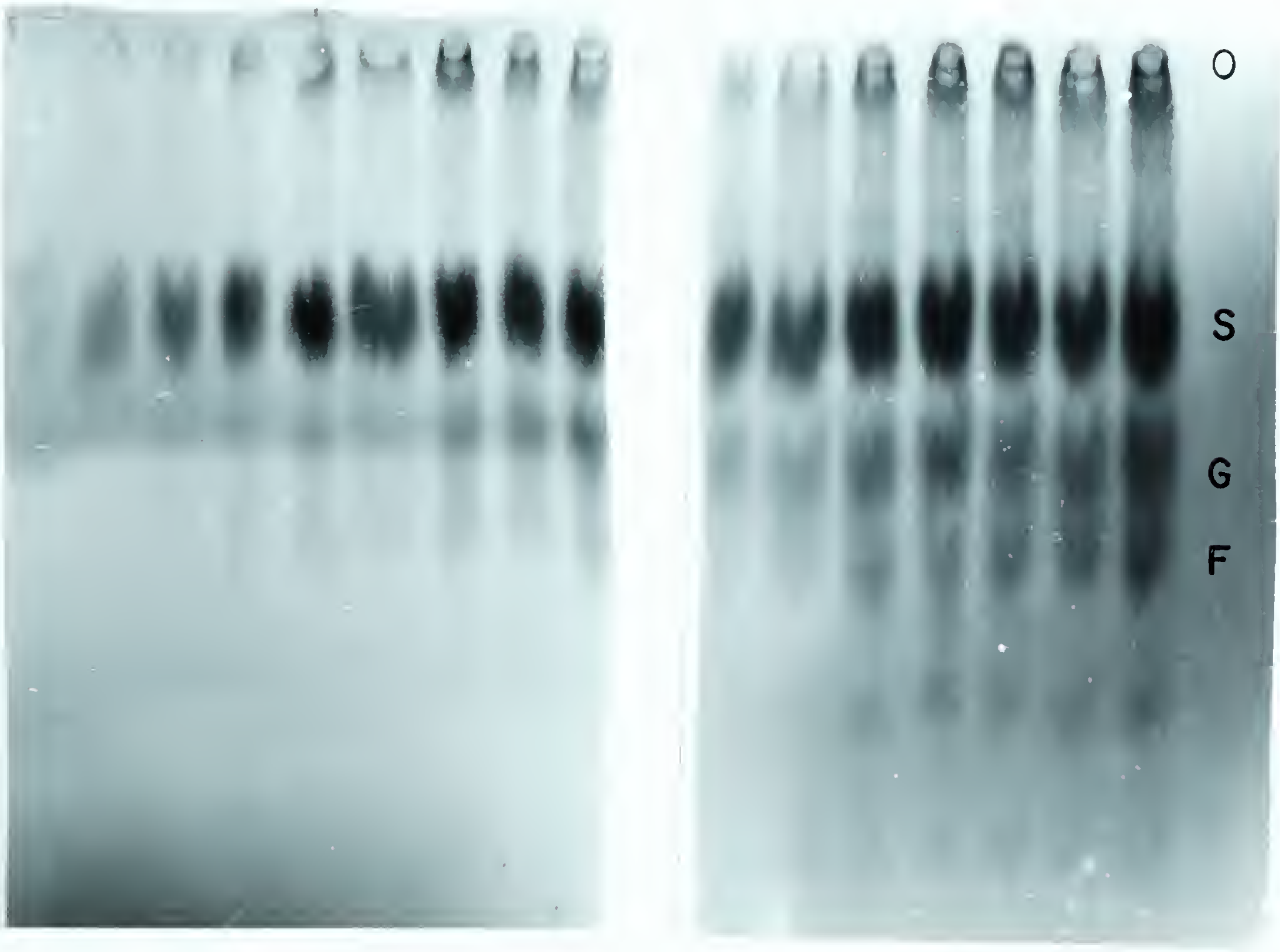


Figure 7. Radioautographs of chromatographs of ethanol-soluble fractions extracted from epicotyls growing on ^{14}C-sucrose. Extracts from left to right were made at the times indicated in Table I; solvent: butanol:acetic acid: water; O = origin; S = sucrose; G = glucose; F = fructose.


O
S
G
F

^{14}C-sucrose and its incorporation into what are presumed to be wall polysaccharides. Apart from a slightly higher concentration of radioactivity at the bases of the internodes, there was no evidence for a localized synthesis of insoluble radioactive material along the shafts of the internodes in growing regions.

2. Growth and Metabolism of Sectioned Epicotyls on Water.

Sections from the third internode were excised as shown in Figure 1 and floated on water for 48 hours. Tables II and III show the growth and weight changes of these sections. Because of lack of substrate, the rate of enlargement was very slow although the growth pattern closely followed that shown in Figure 5 for intact internodes. Only the apical 10 mm of the third internode showed appreciable increases in either fresh weight or length; basal sections did not grow.

The initial ethanol- and ether-insoluble dry weights per 40 sections (Table II), were lower in basal than in apical sections, as was to be expected since the former had fewer and larger cells than the latter (Figure I). After 48 hours incubation on water ,the apical sections had lost up to one quarter of their initial dry weight whereas the basal sections increased in dry weight. Evidently, under these conditins, growth was associated with a loss rather than a gain of insoluble material.

TABLE II

WEIGHTS OF PEA EPICOTYL SECTIONS FLOATED ON WATER FOR 48 HOURS

SECTION	FRESH WEIGHT (mgm/40 sections)		INSOLUBLE DRY WEIGHT (mgm/40 sections)	
	ZERO TIME	48 HOURS	ZERO TIME	48 HOURS
H	112	124	11.0	8.1
1	300	483	16.2	12.5
2	400	558	13.3	12.1
3	416	478	9.4	9.9
4	413	421	8.3	9.0
5	423	448	7.4	8.6

TABLE III

PERCENTAGE CHANGE IN LENGTH AND WEIGHT OF SECTIONS INCUBATED ON WATER FOR 48 HOURS*

SECTION	LENGTH	FRESH WEIGHT	INSOLUBLE DRY WEIGHT
H	-	+11	-26
1	+36	+61	-23
2	+25	+40	- 9
3	+ 2	+15	+ 5
4	0	+ 2	+ 8
5	0	+ 6	+16

*Values calculated from data in Table II as % of initial weights; initial length of sections=5mm.

3. Growth and Metabolism of Sectioned Epicotyls on Sugar.

A. Experiment 1.

(i) Growth: Sections were excised as in Figure 1 and incubated for 24 hours on 2% ^{14}C-sucrose (531,200 cpm in 1.8 ml supplied to 40 sections). The percentage changes in length and fresh and dry weight are shown in Table IV. Growth in length and fresh weight was confined to the hook and apical 10 mm of epicotyl shaft and was much more extensive than growth on water, especially in section one. The insoluble dry weight of all sections increased, the extent ranging from 14 to 24% in the growing regions to 31 to 48% in the maturing regions. There was evidently no proportionality between growth and the increment in dry weight.

(ii) Incorporation of ^{14}C-sucrose: After ethanol and ether extraction, the dried sections were analysed as described in Figure 4 to determine the relative amounts of radioactivity in various fractions. Table V records the amounts of ^{14}C incorporated into Na_2EDTA- and HCl-soluble fractions, the insoluble residue, and the totals for each section. More radioactivity was present in growing than in maturing sections. This was due mainly to a higher incorporation into the HCl-soluble fraction in growing sections. Expressed as a percentage of total insoluble ^{14}C, the growing regions incor-

TABLE IV

PERCENTAGE INCREASE$^+$ IN LENGTH AND WEIGHT OF SECTIONS INCUBATED ON 2% SUCROSE FOR 24 HOURS.*

SECTION	LENGTH	FRESH WEIGHT	DRY WEIGHT
H	-	40	12
1	56	98	14
2	14	24	24
3	4	10	31
4	0	10	48
5	0	4	46

* The basic data for this Table are recorded in Appendix 3.

+ Calculated as % of initial values.

TABLE V

RADIOACTIVITY INCORPORATED INTO MATERIAL INSOLUBLE IN ETHANOL AND ETHER. Sections were incubated on ^{14}C-sucrose for 24 hours. Values given are cpm/40 sections, percentages were calculated as cpm in a fraction x 100/total cpm in all fractions.

SECTION	Na_2EDTA-SOLUBLE		HCl-SOLUBLE		INSOLUBLE RESIDUE		TOTAL
GROWING REGIONS							
H	16,650	30.5%	19,050	44.0%	7,550	25.5%	43,250
1	17,650		29,150		17,750		64,550
2	16,850		27,350		18,150		62,350
MATURING REGIONS							
3	15,650	30.0%	16,950	35.0%	18,000	35.0%	50,600
4	14,100		13,300		16,450		43,850
5	10,350		16,450		12,350		39,150

porated the same proportion of ^{14}C-sucrose into Na_2EDTA-soluble fractions, more into HCl-soluble fractions and less into insoluble residues than maturing regions.

The composition of the radioactive substances synthesized in the three fractions was investigated by chromatography of hydrolysates. Chromatographs, sprayed to reveal carbohydrates, are shown in Figure 8 and radioautographs of chromatographs are shown in Figure 9.

The main component of the hydrolysed Na_2EDTA fraction was identified as galacturonic acid (Figures 8 and 9). Since polyuronides are known to be difficult to hydrolyse beyond the aldobiuronic stage (Jermyn, 1955), the radioactive bands at and near the origin were presumed to be polmers of uronic acid. All of the radioactivity in the original Na_2EDTA fraction was, therefore, considered to be present in pectic acid.

The major radioactive components of the HCl fraction were identified as xylose, arabinose and galactose (Figure 3). The radioactive fraction which remained at the origin (Figure 9) reacted with benzidine (Figure 8) and was presumably an unhydrolysed polysaccharide. Although traces of amino acids were also present, the whole extract will be referred to as hemicellulose.

The residues insoluble in 1N HCl dissolved readily in concentrated H_2SO_4. The main components were identified after hydrolysis as glucose and fructose (Figure 9). The

Figure 8. Chromatographs of hydrolysates derived from section 1 of Na_2EDTA-soluble (A), HCl-soluble (B), and concentrated H_2SO_4-soluble (C) fractions. Spray reagent: benzidine-HCl; solvents: butanol:acetic acid:water (4:1:5 v/v) for (A) and (B), propanol:ethyl acetate:water (7:1:2 v/v) for (C); symbols represent:

a - arabinose
c - unidentified carbohydrate
f - fructose
ga - galactose
gl - glucose
gu - galacturonic acid
u - unidentified
x - xylose

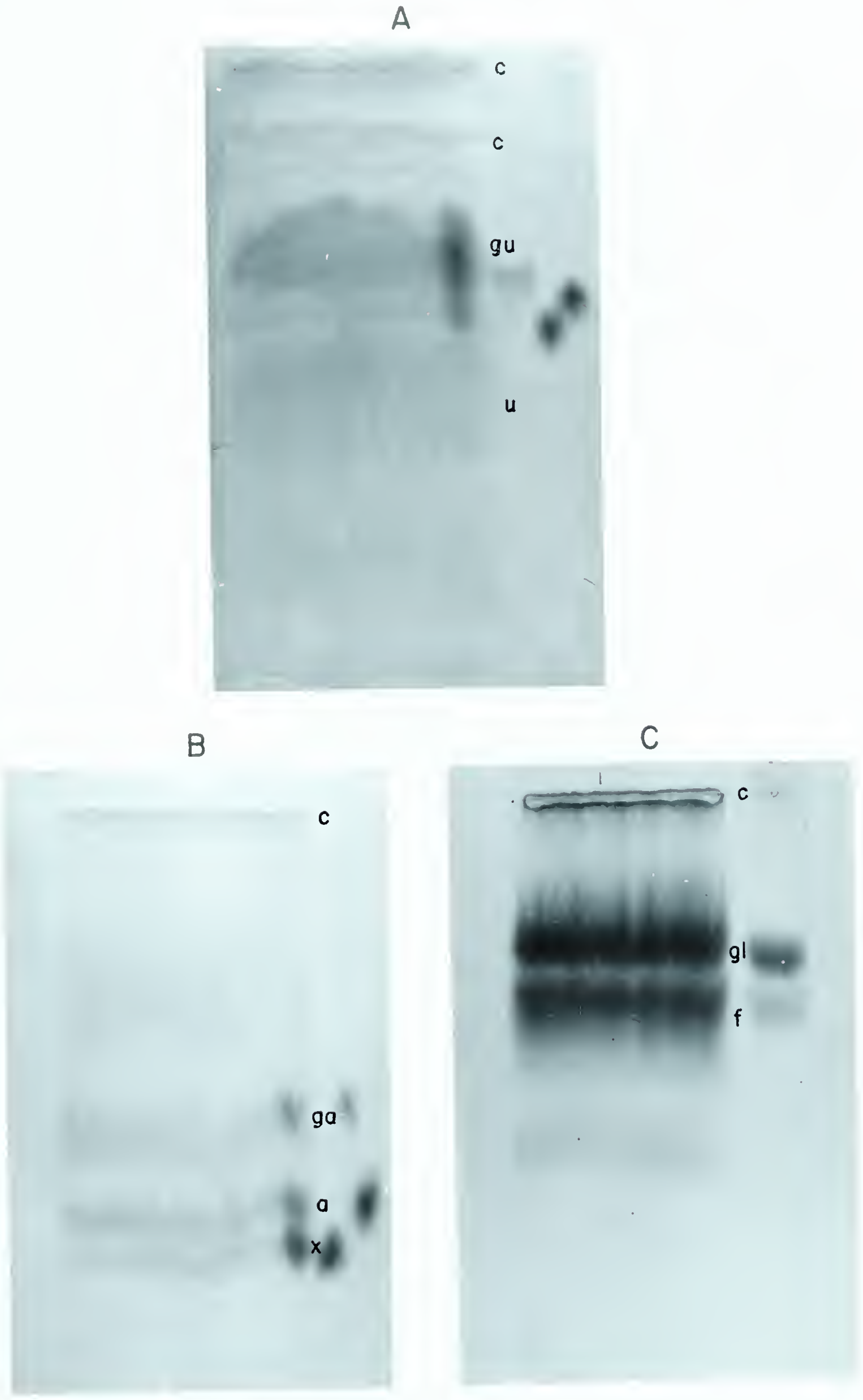
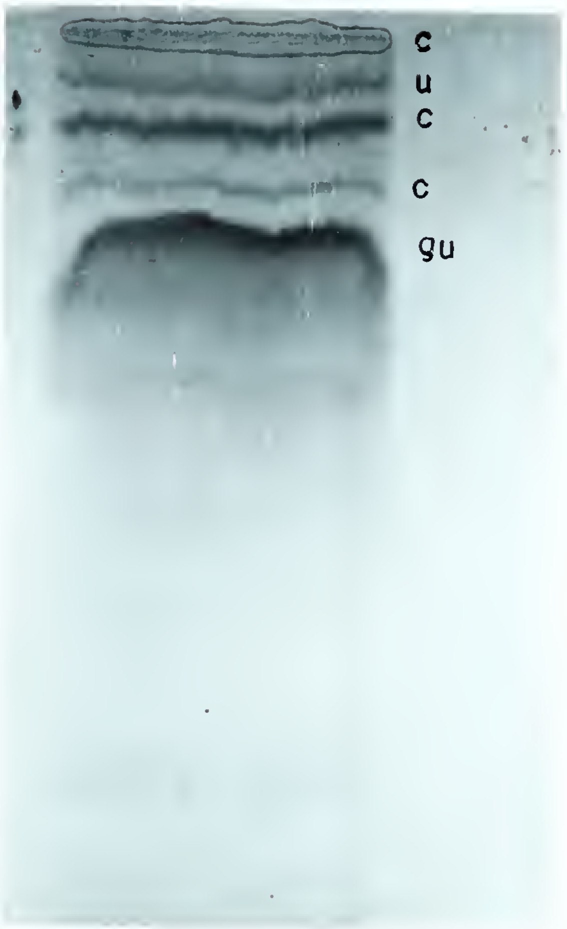

A
c
c
gu
u
B
c
ga
a
x
C
c
gl
f

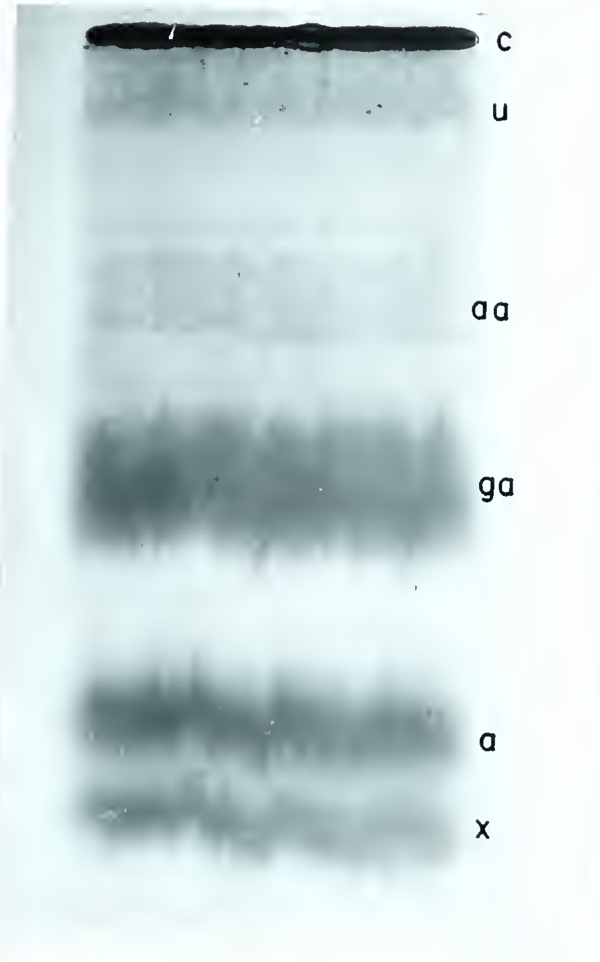
Figure 9. Radioautographs from chromatographs (see Figure 8) of hydrolysates of Na_2EDTA-soluble$^{(A)}$, HCl-soluble $^{(B)}$, and concentrated H_2SO_4-soluble $^{(C)}$ fractions.

Symbols represent; a - arabinose
aa - amino acid
c - unidentified carbohydrate
f - fructose
ga - galactose
gl - glucose
gu - galacturonic acid
p - pentose
u - unidentified
x - xylose

A



B



C

latter was presumably formed from the former since enolisation by $Ba(OH)_2$, added to neutralize H_2SO_4 after hydrolysis, is unavoidable (Bell, 1955). Radioactive material at the origin was polysaccharide in nature. A band of benzidine-reacting carbohydrate with an Rf value corresponding to xylose was not radioactive (cf. Figures 8 and 9) and a band just below the origin which was radioactive did not give a reaction with benzidine. Disregarding these contaminants, the H_2SO_4-soluble fraction will be referred to as cellulose.

The distribution of radioactivity between components of the hemicellulose fraction is shown in Table VI. The growing regions synthesized relatively less xylan, araban, and galactan but more unhydrolysed residue than maturing regions. The latter was the only wall component, the synthesis of which was more extensive in growing than maturing regions. It consisted chiefly of polysaccharide which remained at the origin on chromatographs (Figure 8). Difficulties encountered in eluting it from the paper prevented further analysis.

(iii) Weight Changes: The weight of ^{14}C-sucrose incorporated into wall material can be calculated by dividing total radioactive material (cpm) by the specific activity (cpm/mgm) of the substrate. Uptake of labelled sugar can then be compared to the increase in dry weight of the sections. Table VII shows these calculations made for Experiment 1 from data presented in Table V and Appendix 3.

TABLE VI

RADIOACTIVITY INCORPORATED INTO COMPONENTS OF THE HCl-SOLUBLE FRACTION (SEE TABLE V). Values given are cpm/40 sections, percentages were calculated as cpm in a component x100/total cpm in the HCl-soluble fraction.

SECTION	GALACTOSE		ARABINOSE		XYLOSE		UNHYDROLYSED RESIDUE*	
GROWING REGIONS								
H	5,150	24%	2,200	13%	1,980	10%	9,720	53%
1	7,740		4,150		3,050		14,210	
2	5,030		3,530		2,510		16,280	
MATURING REGIONS								
3	5,150	33%	3,550	20%	2,430	17%	5,820	30%
4	4,410		2,530		2,740		3,620	
5	5,740		3,320		2,820		4,770	

* Calculated as total cpm (Table V) minus recovered cpm.

TABLE VII

COMPARISON OF THE WEIGHT INCREASES OF INSOLUBLE MATERIAL WITH THE WEIGHT INCREASES DUE TO ^{14}C-SUCROSE INCORPORATION. Dry weight increase was calculated from data in Appendix 3; weight of ^{14}C-sucrose incorporated = total cpm (Table V) ÷ specific activity (16,750 cpm/mgm).

	A	B	
SECTION	INCREASE IN INSOLUBLE DRY WEIGHT (mgm/40 sections)	^{14}C-SUCROSE INCORPORATION (mgm/40 sections)	A-B (mgm/40 sections)
H	1.5	2.1	-0.6
1	2.4	3.1	-0.7
2	2.9	3.0	-0.1
3	3.0	2.2	+0.8
4	4.3	2.0	+2.3
5	4.2	1.9	+2.3

In maturing sections, the increase in dry weight of the insoluble fractions more than accounted for the uptake of ^{14}C into these same fractions. The difference could be explained by synthesis from unlabelled endogenous substrates concurrently with synthesis from ^{14}C-sucrose. In the growing sections however, a greater weight of ^{14}C was deposited as wall material than could be accounted for by weight increment. This can only be explained by the breakdown and dissolution of unlabelled materials present in the insoluble fractions of these sections at the beginning of the experiment. Growth of the sections on sucrose is therefore similar to growth on water (Table III) in that it occurs with a loss of initial insoluble material.

B. Experiment 2.

It is clear that the incorporation of ^{14}C-sucrose into the insoluble wall material provides an estimate of wall synthesis which is minimal since it does not include concurrent synthesis from unlabelled endogenous substrates. The supplied sucrose and endogenous materials would mix in a metabolic pool from which substrates would be withdrawn, not only for wall synthesis, but also for respiration. In this case, the specific activity of respired CO_2 and wall substrates would be the same. Amore accurate estimate of

total wall synthesis would then be given by dividing the amount of synthesis from ^{14}C-sucrose by the degree to which respired CO_2 was diluted with unlabelled carbon. Thus, for example, if 6.0 mgm ^{14}C-sucrose were incorporated into the wall and the respired CO_2 as well as the substrates for wall synthesis were 80% as radioactive as the ^{14}C-sucrose, total wall synthesis would equal $6.0 \times 100/80 = 7.5$ mgm.

Accordingly, sections were incubated on radioactive sucrose as in Experiment 1 and measurements were made of the specific activity of CO_2 respired during the incubation. For each of the six sections, duplicate flasks (A and B) containing 40 sections each were carried through the experiment. The initial and final fresh and dry weights of the sections are given in Appendices 4 and 5. Calculations for wall synthesis from ^{14}C-sucrose and the differences between synthesis and weight increment (minimal breakdown) are shown in Table VIII. Table IX shows the specific activities of respired CO_2 relative to that of the supplied ^{14}C-sucrose, together with the calculations made from these values for wall synthesis from all substrates and breakdown of insoluble materials.

Growth of the sections was confined to the hook and apical 10 mm of the epicotyl (Appendix 5) and dry weight increments were approximately the same in all

TABLE VIII

WALL SYNTHESIS FROM ^{14}C-SUCROSE AND BREAKDOWN OF PRE-EXISTING INSOLUBLE MATERIALS.

A and B are duplicates.

SECTION	SYNTHESIS mgm/40 sections*		MINIMAL BREAKDOWN mgm/40 sections+	
	A	B	A	B
H	4.2	4.1	1.7	1.3
1	6.4	5.9	3.2	3.1
2	4.6	4.3	2.8	2.3
3	3.6	3.9	1.0	1.0
4	2.1	2.9	1.0	0.6
5	2.3	3.0	0.4	0

* Calculated as $\dfrac{\text{cpm in insoluble fraction}}{\text{specific activity } ^{14}\text{C-sucrose (1990 cpm/mgm)}}$.

+ Initial dry weight (recorded in Appendix 4) + wall synthesis from ^{14}C-sucrose - final dry weight (Appendix 4).

TABLE IX

WALL SYNTHESIS AND BREAKDOWN OF INSOLUBLE MATERIALS, TAKING INTO ACCOUNT POSSIBLE ADDITIONAL SYNTHESIS FROM UNLABELLED SUBSTRATES. A and B are duplicate determinations.

SECTION	RELATIVE SPECIFIC ACTIVITY CO$_2$ $\frac{\text{cpm/um CO}_2\text{ x100}}{\text{cpm/umC in }^{14}\text{C-sucrose}}$		SYNTHESIS FROM LABELLED PLUS UNLABELLED SUBSTRATE* (mgm/40 sections)		BREAKDOWN+ (mgm/40 sections)	
	A	B	A	B	A	B
H	82	76	5.1	5.4	2.6	2.6
1	81	76	7.9	7.8	4.7	5.0
2	78	69	5.9	6.2	4.1	4.2
3	76	67	4.7	5.8	2.1	2.9
4	61	65	3.4	4.5	2.3	2.2
5	64	68	3.6	4.4	1.7	1.4

* Wall synthesis from ^{14}C-sucrose (Table VIII) x I/R.S.A. CO$_2$

+ Initial dry weight (Appendix 4) + calculated wall synthesis from all substrates - final dry weight (Appendix 4).

sections (average of 2.6 mgm/40 sections, Appendix 4). Wall synthesis from ^{14}C-sucrose was 1.6 to 2.4 times greater than the weight increment in growing sections (Table VIII). In section one, for example, about 6 mgm ^{14}C-sucrose was incorporated into wall materials but at the same time the increase in weight amounted to only 3 mgm. Thus at least 3 mgm of the original insoluble material dissolved during the experiment. In basal sections the discrepancies between synthesis from ^{14}C-sucrose and weight increment were much less than in apical sections. This implies that the dissolution of insoluble materials occurred mainly in growing tissue.

Respired CO_2 was 80-70% as radioactive as supplied ^{14}C-sucrose in apical sections and 70-60% as radioactive in basal sections (Table IX). Evidently considerable amounts of unlabelled substrate were available endogenously for respiration, and presumably also for wall synthesis in all sections. If wall synthesis occurred from substrates at the same specific activity as respired CO_2, it would have been two to three times greater than weight increment in the apical sections and 1.5 to two times greater in basal sections (Table IX). This does not change the conclusion that dissolution of insoluble material was greatest in those sections which grew most but it does imply that some dissolution may occur in tissue which does not expand.

DISCUSSION

Results of the present studies lead to the conclusion that the growth of pea epicotyl tissue does not depend on cell wall synthesis. The insoluble material of tissue floated on 2% sugar increased in amount but the increase in growing sections was either the same as (Appendix 4) or less than (Table VII) the increase in maturing sections. This was true whether the weight increase was calculated as percentage of initial dry weight or as increment per section. When the supplied sugar was radioactive, virtually all of the insoluble products formed from it were wall carbohydrates (Figures 8 and 9). The total weights of these carbohydrates were higher in growing sections than in mature sections (Tables VII and VIII). However, when expressed as a percentage of initial dry weight (Table X), wall synthesis was approximately the same in all sections whether they grew or not.

The only wall component which was synthesized in greater proportions in growing than in maturing sections was part of the hemicellulose fraction (Tables V and VI). Little cellulose was synthesized in sections that grew and pectin synthesis was approximately the same in all sections (Table V). Similar results were obtained by Northcote (1961) for the composition of wall materials deposited in growing and maturing vascular tissue of pine. Using corn roots,

TABLE X

SYNTHESIS OF INSOLUBLE MATERIALS, EXPRESSED AS PERCENTAGE OF INITIAL INSOLUBLE MATERIAL*

SECTION	EXPERIMENT 1 (Synthesis from labelled substrates only)	EXPERIMENT 2 (Synthesis from labelled substrates only)	EXPERIMENT 2 (Synthesis from labelled plus unlabelled substrates)
H	16	35	43
1	16	44	57
2	20	34	46
3	17	40	55
4	15	30	47
5	15	35	53

*Values for Experiment 1 were calculated from data given in Table VII and Appendix 3, and values for Experiment 2 from Tables VIII and IX and Appendix 4.

Lund et al., (1958) showed that cellulosewas deposited in increasing amounts with maturation. Both Jensen et al., (1960) and Bishop et al., (1958) found that hemicelluloses were preponderant in wall material of young growing tissues. It is possible that hemicelluloses have some special function in cell enlargement but it is difficult to imagine their synthesis as a cause of growth when they are formed in considerable amounts in tissues which do not expand. Their role may be simply to provide support and protection to young cells without impeding wall extension.

The dissolution of insoluble materials in growing sections was demonstrated directly with sections incubated on water (Table II) andindirectly with sections incubated on sucrose (Tables VII and VIII). The insoluble fractions of all sections contained mainly wall polysaccharides and nitrogenous materials (protein, nucleic acids, etc.). The amounts of the latter actually increase during growth of pea epicotyl sections, even on water (Christiansen and Thimann, 1951; Thimann, 1954). Therefore, there can be no doubt that the losses in insoluble weight reflect breakdown of wall polysaccharides.

It should be noted that the present methods measured only breakdown and dissolution of the pre-existing wall and did not include decorporation of radioactive wall materials synthesized during the experiments. This would undoubtedly occur. Several authors have supplied labelled

substrates to young growing tissues for a brief period, followed by a longer period on water, and measured the uptake and subsequent loss of label from wall materials. Ordin _et al._, (1955) found no significant loss of label using oat coleoptiles supplied with ^{14}C-glucose but losses were established from pectic materials synthesized from $^{14}CH_3$-methionine. Matchett and Nance (1962) repeated the experiment with pea epicotyls supplied with ^{14}C-glucose and found clear losses from all wall fractions, mainly pectin, but also including hemicelluloses and cellulose. These findings were similar to those of Margerie and Lenoël (1961) who demonstrated decorporation from hemicellulose and cellulose in wheat roots.

Only one report has been found in the literature of a loss of wall weight during growth. Solberg and Higinbotham (1957) presented data for the growth of pea epicotyl sections on water for six days from which it was possible to calculate that insoluble dry weight fell by 35%. These authors did not themselves point this fact out. It is generally agreed that wall materials are usually stable in mature plant tissues (Whistler & Sannella, 1960) but this may not be true in young tissues. Esau (1958) has cited several instances which may be examples of cell wall digestion _in situ_. Thick walls occur in the endosperm of date palm (_Phoenix dactylifera_), persimmon (_Diospyros_), _Asparagus_, and _Coffea arabica_. These walls become thinner during germination, indicating the presence of both hemicellulases and cellulases. There are a

few reports (Gascoigne and Gascoigne, 1960; Macleod and Sandie, 1961) of the isolation of such enzymes from young tissues. Also, pectinases have been extracted from higher plant tissues which de-differentiate, e.g., during abscission (Yager, 1960).

In the present studies, dissolution of wall materials was more pronounced in growing than in maturing regions. Dissolution of at least one quarter of the original insoluble material occurred in all experiments (Tables III, VII, VIII) in the hook and in section one. In basal sections there appeared to be little or no dissolution. However, in Experiment 2, when breakdown was calculated taking into account synthesis from unlabelled endogenous substrates (Table IX), it was substantial in the basal sections, amounting to 1/3 to 1/2 of that in apical sections. Breakdown in all sections would be even more extensive if account were taken of turnover of radioactive wall. For these reasons, it is probable that the dissolution of wall materials is not confined to growing tissues.

As the main conclusion of this work, it is proposed that the breakdown and dissolution of the older part of the primary wall in growing epicotyl sections could allow re-arrangement and loosening of that material which constricts growth. There is no evidence that the wall synthesis that normally accompanies growth is the driving force behind cell elongation, although there may

be more or less of certain components formed in growing than in maturing tissue. Assuming that wall plasticity is the limiting factor of growth, the possibility may now be envisaged that it is brought about by the metabolic turnover of some or all components of the wall.

BIBLIOGRAPHY

1. Adamson, D. H. and Adamson, H., 1958. Auxin action on coleoptiles in the presence of nitrogen at low temperature, Science, 128, 532-533.

2. Bannan, M. W., 1956. Some aspects of the elongation of fusiform cambial cells in *Thuja occidentalis* L., Can. J. Bot., 34, 175-196.

3. Bayley, S. T., Colvin, J. R., Cooper, F. P. and Martin-Smith, Cecily A., 1957. The structure of the primary epidermal cell wall of *Avena* coleoptiles, J. Biophys. Biochem. Cytol., 3, #2, 171-182.

4. Bell, D. J., 1955. Mono- and oligosaccharides and acidic monosaccharide derivatives, Moderne Methoden Der Pflanzenanalyse, 2. (ed. K. Peach and M. V. Tracey).

5. Bennet-Clark, T., 1956. Salt accumulation and mode of auxin action, A preliminary hypothesis, Symposium on Plant Growth Substances, 284-291. (Butterworths, ed. R. L. Wain and F. Wightman).

6. ---------------- , 1961. in Fourth International Conference on Plant Growth Regulation (Iowa State Univ. Press) p. 328.

7. Bishop, C. T., Bayley, S. T. and Setterfield, G., 1958. Chemical constitution of the primary cell walls of *Avena* coleoptiles, Pl. Physiol., 33, #4, 283-289.

8. Block, R. J., Durrum, E. L., and Zweig, G., 1952. Paper Chromatography, (Academic Press, New York.)

9. Bonner, J., 1934. Studies on the growth hormones of plants. V. The relation of cell elongation to cell wall formation, Proc. Nat. Acad. Sci. U. S. A., 20, 393-397.

10. ---------- , 1950. Plant Biochemistry (Academic Press, New York).

11. ---------- , 1961. On the mechanisms of auxin-induced growth, in Plant Growth Regulation, 4th International Congress (Iowa State Univ. Press) p. 307-328.

12. Boroughs, H. and Bonner, J., 1953. Effects of indoleacetic acid on metabolic pathways, Arch. Biochem. Biophys., 46, 279-290.

13. Brown, R., 1959. The regulation of growth and differentiation in the root. In Biochemistry of Morphogenesis; Proc. 4th International Congress of Biochemistry, 6, p. 77.

14. --------- , and Neish, A. C., 1954. The biosynthesis of cell wall carbohydrates, Glucose-C^{14} as a cellulose precursor in plants, Can. J. Biochem. and Physiol., 32, 170-177.

15. Bryan, W. H. and Newcombe, E. H., 1954. Stimulation of pectin methylesterase activity of cultured tobacco pith by indoleacetic acid, Physiol. Plant., 7, 290-297.

16. Carlier, A. and Buffel, K., 1955. Polysaccharide changes in the cell walls of water absorbing potato tuber tissue in relation to auxin action, Acta Bot. Neérl., 4, 551-564.

17. Carr, D. J. and Ng, E. K., 1959. The sequestration of calcium from preparations of wheat coleoptile cell walls, Physiol. Plantarum, 12, 264-274.

18. Christiansen, G. S. and Thimann, K. V., 1950. The metabolism of stem tissue during growth and its inhibition, III. Nitrogen Metabolism, Arch. Bioch., 28, 117.

19. Cleland, R., 1960. Auxin-induced methylation in maize, Nature, *185*, 44.

20. ----------- , and Bonner, J., 1956. The residual effect of auxin on the cell wall, Pl. Physiol., *31*, 350-354.

21. Cormack, R. G. H., 1956. A further study on the growth of *Brassica* roots in solutions of pectic enzymes, Can. J. Bot., *34*, 983-987.

22. ------------------, 1961. The development of root hairs, Recent Adv. in Bot., section 8, 812-815.

23. Frey-Wyssling, A., 1952. Growth of plant cell walls, Symposia Soc. Exptl. Biol., *6*, 320-328.

24. ----------------- , and Stecher, H., 1951. Das Flachenwachstum der Pflanzlichen Zellwande, Experientia, *7*, 420-421.

25. ----------------- , 1957. Macromolecules in Cell Structure, (Harvard Univ. Press, Cambridge.)

26. Galston, A. W. and Purves, W. K., 1960. The mechanism of action of auxin, Ann. Rev. Pl. Phys., *11*, 239-276.

27. Gascoigne, J. A. and Gascoigne, Margaret M., 1960. Biological Degradation of Cellulose (Butterworth, London) pp. 56, 243.

28. Glaziou, K. T., 1959. The localization and properties of pectin methylesterase of *Avena* coleoptiles, Physiol. Plant., *12*, 670-680.

29. Green, P. B., 1960. Multinet growth in the cell wall of *Nitella*, J. Biophys. Biochem. Cytol., *7*, #2, 289-296.

30. Hayward, H. E., 1938. The Structure of Economic Plants (The Macmillan Co.) pp. 354-356.

31. Heath, O. V. S. and Clark, J. E., 1956. Chelating agents as plant growth substances, Nature, 177, 1118-1121.

32. Heyn, A. N. J., 1940. The physiology of cell elongation, Bot. Rev., 6, #10, 515-574.

33. Jansen, E. F., Jang, R., Albersheim, P., and Bonner, J., 1960. Pectic metabolism of growing cell walls, Plant. Physiol., 35, 87-97.

34. Jensen, W. A. and Ashton, Mary, 1960. Composition of the developing primary wall in onion root tip cells, I. Quantitative analyses, Pl. Physiol., 35, #3, 313-323.

35. Jermyn, M. A., 1955. Cellulose and hemicelluloses, Moderne Methoden der Pflanzenanalyse vol. 2, (Springer-Verlag, ed. K. Peach and M. V. Tracey) 197-225.

36. Kertesz, Z. I., 1951. The Pectic Substances (Interscience Publishers, Inc., New York, N. Y.,).

37. Lund, H. A., Vatter, A. E., and Hanson, J. B., 1958. Biochemical and cytological changes accompanying growth and differentiation in *Zea mays*, J. Biophys. Biochem. Cytol., 4, 87-98.

38. Macleod, A. M. and Sandie, R., 1961. Cell wall metabolism, I. Hemicelluloses of *Bromus* seeds, New Phytol., 60, 117-128.

39. Margerie, C. et Lenoël, C. P., 1961. Cinetique de biosynthèses de la cellulose, Biochim. Biophys. Acta, 47, 275-287.

40. Matchett, W. H. and Nance, J. F., 1962. Cell wall breakdown and growth in pea seedling stems, Amer. J. Bot., 49, #4, 311-319.

41. Mortimer, D. C. and Wylam, Clare B., 1962. The incorporation of ^{14}C into cellulose and other polysaccharides of sugar beet leaf during short term photosynthesis in $^{14}CO_2$, Can. J. Bot., 40, 1-11.

42. Mühlethaler, K., 1950. Elektronenmikroskopische Untersuchungen über den Feinbou und das Wachstum der Zellmembranen in Mais-und Haferkoleoptilen, Ber. schweiz. bot. Ges., 60, 614-628.

43. Nance, J. F., 1958. Effects of indoleacetic acid on the utilization of acetate-1-C^{14} by pea stem slices, Plant Physiol., 33, 93-98.

44. Northcote, D. H., 1958. The cell walls of higher plants: Their composition, structure, and growth, Biol. Rev. Camb. Phil. Soc., 33, 53-102.

45. ---------------- , 1961. Changes in the chemical composition of a cambial cell during its differentiation into xylem and phloem tissue in trees, I. Main components, Bioch. J., 81, 449-455.

46. Ordin, L. and Bonner, J., 1957. Effect of galactose on growth and metabolism of Avena coleoptile sections, Pl. Physiol., 32, 212-215.

47. -------- , Cleland, R., and Bonner, J., 1955. Influence of auxin on cell wall metabolism, Proc. Natl. Acad. Sci.

U. S., 41, 1023-1029.

48. ---------- , -------------------- , 1957. Methyl esterification of cell wall constituents under the influence of auxin, Pl. Physiol., 32, 207-212.

49. Porter, H. K. and May, L. H., 1954. Metabolism of radioactive sugars by tobacco leaf discs, J. Exp. Bot., 6, #16, 43-63.

50. Preston, R. D. and Ripley, G. W., 1954. An electron-microscopic investigation of the walls of conifer cambium, J. Exptl. Bot., 5, 409-412.

51. -------------- , 1958. The physiological significance of electron microscopic investigations of plant cell walls, Sci. Progr., 46, 593-605.

52. Ray, P. M., 1961. Problems in the biophysics of growth, in Fourth International Conference on Plant Growth Regulation (Iowa State Univ. Press) 381-385.

53. Roelofsen, P. A., 1959. The plant cell wall. Handbuch der Pflanzenanatomie, Band III,Teil 4 (Gebruder Borntraeger, Berlin, ed. W. Zimmerman and F. G. Czenda).

54. ----------------- and Houwink, A. L., 1953. Architecture and growth of the primary cell wall in some plant hairs and in the *Phycomyces* sporangiophore, Acta Botan. Neérl., 2, 218-225.

55. Scott, F. M., Hamner, K. C., Baker, E., and Bowler, E., 1956. Electron microscope studies of cell wall growth in the onion root, Am. J. Bot., 43, 313-324.

56. Setterfield, G. and Bayley, S. T., 1957. Studies on the

mechanism of deposition and extension of primary cell walls, Can. J. Bot., 35, 435-444.

57. ------------------ --- ------------ , 1959. Deposition of cell walls in oat coleoptiles, Can. J. Bot., 37, 861-870.

58. ------------------ --- ------------ , 1961. Structure and physiology of cell walls, Ann. Rev. Pl. Physiol., 12, 35-62.

59. Solberg, R. A. and Higinbotham, N., 1957. The effects of IAA, sucrose and eugenol on differentiation in segments of etiolated pea epicotyls, Amer. J. Bot., 44, 769-778.

60. Tagawa, T. and Bonner, J., 1957. Mechanical properties of the *Avena* coleoptile as related to auxin and to ionic interactions, Pl. Physiol., 32, 207-212.

61. Thimann, K. V., 1954. The physiology of growth in plant tissues, Amer. Scientist, 42, #4, 589-606.

62. Wardrop, A. B., 1962. Cell wall organization in higher plants I. The primary wall, Bot. Rev., 28, #2, 241-285.

63. ------------- , 1955. The mechanism of surface growth in parenchyma of *Avena* coleoptiles, Australian J. Bot., 3, 137-148.

64. ------------- , 1956. The mechanism of surface growth in the parenchyma of *Avena* coleoptiles, Bioch. Biophys. Acta, 21, 200-201.

65. Went, F. W. and Thimann, K. V., 1937. Phytohormones (The Macmillan Co.)

66. Whistler, R. L. and Sannella, J. L., 1958. Proceedings of

the IVth International Congress of Biochemistry, Carbohydrate chemistry of substances of biological interest, Vol. 1, Symp. 1 (Pergamon Press, London) p. 1.

67. Yager, R. E., 1960. Possible role of pectic enzymes in abscission, Pl. Physiol., 35, #2, 157-162.

APPENDIX I.

PROCEDURE FOR THE PREPARATION AND PHOTOMICROSCOPY OF SLIDES.

Tissue from the apical region of etiolated pea epicotyls was sectioned as illustrated in Figure 1; fixed in F. A. A.; dehydrated with a standard butyl alcohol series and stained with Erythrocin B. The dehydrated tissue was then embedded in Fisher Tissue Mat (m.p. 56.5°C.); cut at 12 microns with a rotary microtome; and using a standard method, permanently mounted on microscope slides. The following staining procedure was used:

a.) Absolute Xylene
b.) 50/50 Absolute Xylene/Absolute Ethanol
c.) 95% Ethanol
d.) 85% Ethanol
e.) 70% Ethanol
f.) Safranin in 70% Ethanol
g.) 85% Ethanol
h.) 95% Ethanol
i.) Fast Green in 95% Ethanol
j.) Absolute Ethanol
k.) 50/50 Absolute Xylene/Absolute Ethanol
l.) Absolute Xylene

Finally, the tissue was mounted in Permount (Fisher Scientific Company).

All photographs of the slides were taken with a Zeiss Photomicroscope (55575) using Kodak Panatomic-X Film (FX 135-20).

APPENDIX 2

ELONGATION OF APICAL REGIONS OF DETACHED PEA EPICOTYLS (see Figure 5)

	INITIAL LENGTH	AVERAGE INCREASE IN LENGTH	FINAL LENGTH / INITIAL "	% SEGMENTAL INCREASE
1	2mm	2.0mm	2.00	100
2	"	2.2	2.08	109
3	"	2.0	2.00	100
4	"	1.7	1.84	34
5	"	0.7	1.34	34
6	"	0.5	1.23	11
7	4mm	0.2	1.06	5
8	"	0.2	1.04	4
9	"	0.1	1.03	3
10	"	0.1	1.02	2
11	"	0.1	1.02	2
12	"	0.1	1.02	2
13	3-4mm	0.1	1.02	5

APPENDIX 3

LENGTH AND WEIGHT CHANGES OF SECTIONS INCUBATED ON 2% SUCROSE (see Table IV)

SECTION	LENGTH (mm/ section)		FRESH WEIGHT (mgm/40 sections)		DRY WEIGHT (mgm/40 sections)	
	ZERO TIME	24 HOURS	ZERO TIME	24 HOURS	ZERO TIME	24 HOURS
H	—	—	131	184	12.0	13.4
1	5.0	7.8	340	673	16.6	19.0
2	"	5.7	475	588	12.3	15.2
3	"	5.2	473	518	9.7	12.7
4	"	5.0	499	540	8.9	13.2
5	"	5.0	547	571	8.9	13.0

APPENDIX 4

DRY WEIGHT CHANGES DURING INCUBATION OF SECTIONS ON ^{14}C-SUCROSE (see TABLES VIII and IX). A and B are duplicate determinations.

SECTION	ETHANOL- AND ETHER-INSOLUBLE DRY WEIGHT (mgm/40 sections)		
	ZERO TIME	24 HOURS	
		A	B
H	12.1	14.6	14.9
1	13.9	17.1	16.7
2	13.1	14.9	15.1
3	9.6	12.2	12.5
4	8.4	9.5	10.7
5	7.6	9.5	10.6

APPENDIX 5

FRESH WEIGHT CHANGES DURING INCUBATION OF SECTIONS ON ^{14}C-SUCROSE.

A and B are duplicate determinations.

SECTION	ZERO TIME (mgm/40 sections)	24 HOURS (mgm/40 sections)		% CHANGE*
		A	B	
H	133	195	212	+ 54
1	293	555	650	+106
2	385	480	522	+ 30
3	394	408	461	+ 10
4	397	395	428	+ 4
5	445	388	455	- 5

*Calculated as percentage of zero time fresh weight.

[illegible]

[illegible]

[illegible]	[illegible]		[illegible]	[illegible]
	[illegible]	[illegible]		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

B29803